

REMARKS

Applicants note with appreciation that the examiner has allowed claims 1, 2 and 4. Applicants now have amended those claim 1 and claim 5 to exclude the compounds PCIH and PCBH originally within the scope of the claims. These amendments are made in view of the teachings of PCT application WO86/04582, previously made of record. This PCT application relates to compounds for the killing of internal parasites in animals and does not have any relevance to the methods of treatment of the present invention.

Claim 10 has been amended to place it in independent form.

Prior to addressing the specific concerns regarding claims 5-6, 8-10 and 12-14 that the examiner set forth in this Office Action, Applicants provide below general background information on iron overload, iron toxicity and chelating agents.

Excess iron can occur in humans for a number of reasons, including the presence of hemolytic anemia, hereditary or secondary hemochromatosis or hepatitis. In addition, regular blood transfusions administered to treat anemia can cause the body to retain too much iron. As one example, iron overload occurs in people with sickle cell syndromes who have required numerous red cell transfusion. The body's iron stores become saturated after receiving approximately 500 mg Fe/kg, or 20-30

transfusions. Progressive iron accumulation beyond this point will lead to organ toxicity, particularly to the heart, liver and endocrine organs.

Iron overload can cause microbial infection, cardiomyopathy, arthropathy, neoplasia and certain endocrine disorders. According to the website of the Iron Overload Disease Association, excess iron also lowers the immune system and has been found to trigger or exacerbate Alzheimer's disease, multiple sclerosis and Parkinson's disease, among other conditions.

The goal of iron chelation therapy is to prevent iron-mediated injury to cells. The basis of this injury is the very property that makes iron vital to all life: it can exist in either of two stable oxidation states. Iron ions in aqueous solution exist either in the ferrous (Fe^{2+}) state or the ferric (Fe^{3+}) state. The shift of electrons between iron and donor molecules is the basis of energy production by controlled oxidation of carbohydrates, proteins and lipids. Iron is a key element in most of the cytochrome enzymes involved in the oxidative phosphorylation of the Krebs cycle. Because of its ability to participate in chemical reactions that involve the shift of electrons between molecules (reduction-oxidation or redox reactions), the body tightly regulates iron. When iron is tightly bound to a chelator molecule, whether a protein or a

small chemical, the reactivity of the iron is greatly dampened. The key iron storage protein in the body is ferritin, which is a very large spherical molecule. Iron is deposited as semi-crystalline deposits inside these protein "vaults," and iron that is sequestered within ferritin is metabolically inactive.

The iron deposits in patients who have received multiple blood transfusions from chronic anemia, such as thalassemia, can exceed the storage and detoxification capacity of ferritin. Consequently, "free" (or, more accurately, loosely bound) iron begins to accumulate in tissues and blood. This "free" iron can catalyze the formation of very injurious compounds, such as hydrogen peroxide, which are normal metabolic by-products (Fenton reaction).

The hydroxyl radical is highly reactive, and attacks lipids, proteins and DNA. The initial reaction with each of these molecules is the formation of peroxides (e.g., lipid peroxides) that can interact with other molecules to form cross-links. These cross-linked molecules perform their normal functions either poorly or not at all.

Iron chelators protect cells from iron-mediated toxicity in two ways: by removal of excess iron and by neutralization of "free" iron.

Many chelators are used in chemistry and industry; however, only a few are clinically useful since most have dangerous side effects. Currently, Desferal™, which comprises desferrioxamine (DFO), is the only hyperferremic drug available for chelation therapy of iron overload. The problem with this drug is that it must be administered intravenously, and its side effects include hypotension, growth retardation and neurological effects.

According to the Cooley's Anemia Foundation web site (downloaded pages of which are attached to this response), this method of chelation therapy involves infusing the drug through a small battery-operated pump, worn under the skin of the stomach or legs, five to seven times a week for up to 12 hours. The web site states that this treatment method is so difficult that many patients do not keep up with the therapy-a decision that obviously can worsen a patient's condition and prove fatal.

Accordingly, the synthesis and identification of novel iron chelators for *in vivo* use that are effective, non-toxic and preferably can be administered orally is a most valuable invention. As outlined in the specification and in the Declaration provided herewith, the present inventors have identified specific classes of PCIH analogs which satisfy these valuable characteristics.

Turning now to the outstanding Office Action, the examiner has maintained her earlier rejection of claims 5, 6 and 8-14 under 35 U.S.C. §112, first paragraph, on the basis that the specification does not reasonably provide enablement for all overload diseases, including Friedreich's ataxia and β -thalassemia. In particular, the examiner asserted that the specification does not give guidance that the *in vitro* data provided would correlate with *in vitro* settings, particularly in terms of treating the diseases claimed. This rejection is traversed.

Applicants provide herewith a Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Des Richardson, one of the inventors of the invention described and claimed in the present application. In this declaration, Dr. Richardson provides details of additional *in vitro* experiments which support the claims that selected PCIH analogues are effective iron chelating agents. He further describes *in vivo* experiments, the results of which illustrate that selected PCIH analogues as claimed are effective iron chelators *in vivo* and are orally active. Applicants respectfully submit that these data clearly demonstrate that the selected PCIH analogues are suitable for use *in vivo* as iron chelating agents. One of ordinary skill in the art would believe from reading the specification iron chelating agents having the demonstrated

characteristics can be suitable for use in patients suffering from iron overload and have certain advantages over currently used iron chelating agents, such as DFO.

The examiner made a point of stating that PCIH analogs "do not have beneficial effects" on Friedreich's ataxia patients, citing Richardson, *Expert Opinion on Investigational Drugs*, and that DFO as an iron chelator has been found not to be an effective treatment for thalassemia. She further asserted that the use of Fe chelators for the treatment of β -thalassemia and Friedreich's ataxia is "challenging owing to the potential of these compounds to cause toxicity," citing Richardson, *Am. J. Hematol.* 73:200-210 (2003). As Dr. Richardson points out in his declaration, these references, published after the priority date of this application, are from his research lab. The *Am. J. Hematol.* paper refers to PCIH analogs, providing:

[t]hese tridentate ligands bind Fe(III) [99] and have shown similar chelation efficiency to PIH and low toxicity *in vitro* [98]. Importantly, this class of chelators can access mitochondrial Fe pools, offering a potential treatment for FA [7]. Further *in vivo* studies are under way to determine their efficacy.

Contrary to the examiner's assertion, applicants respectfully submit that this reference clearly supports the assertion that

PCIH analogs, as described above, can provide beneficial effects for FA patients. As Dr. Richardson notes in his declaration, further *in vivo* studies in his lab indeed have supported that fact.

Furthermore, the other reference cited by the examiner, *Expert Opinion on Investigational Drugs*," discusses the potential of iron chelators, namely the PCIH analogs, as agents to remove mitochondrial iron deposits. As Dr. Richardson explains in his declaration, these PCIH iron chelators have been specifically designed to enter and target mitochondrial iron pools, which is a property lacking in DFO, the only chelator currently in widespread clinical use. Standard chelation regimens (DFO) may not work in Friedreich's ataxia (FA) patients because the drugs are hydrophilic and cannot cross the mitochondrial membrane. In contrast, however, PCIH iron chelators preferably comprise a hydrophobic heterocyclic or aromatic group and can be highly effective as ligands for mobilizing mitochondrial non-heme Fe from reticulocytes. In particular, as Dr. Richardson states, PCIH analogs such as PCTH and PCIH can cross the mitochondrial membrane and mobilize mitochondrial Fe pools, thereby overcoming

the disadvantage of DFO which cannot effectively deplete Fe from this compartment.

Applicants respectfully submit that they have shown that their studies have identified PCIH analogs having desired iron chelating properties both *in vitro* and *in vivo*. They further respectfully submit that all of the pending claims now are in condition for allowance.

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